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Nakiterpiosin, a novel cytotoxic C-nor-D-homosteroid from the Okinawan sponge *Terpios hoshinota*

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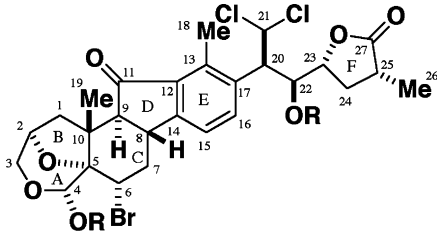
Abstract—A novel cytotoxic compound, nakiterpiosin (**1**), was isolated from the Okinawan sponge *Terpios hoshinota*. Its structure was determined by spectroscopic analysis. The absolute stereostructure of **1** was also determined by a modified Mosher's method. Nakiterpiosin showed potent cytotoxicity against P388 cells.

The overgrowth of hard-coral communities by other organisms has recognized dramatic decline in such corals.[1] It has been reported that these organisms produce toxic compounds; for example, the steroidal alkaloids plakinamines A and B have been isolated from the marine sponge *Plakina* sp., which overgrows coral heads.[2] Nakienones A–C and nakitriol were isolated from the cyanobacteria *Synechocystis* sp., which also overgrows coral reefs.[3] We focused on the overgrowth of coral communities by several large zones of *Terpios hoshinota* in the waters off the Okinawan villages of Nakijin and Yomitan. Recently, we reported the isolation and structure determination of terpiodiene from the Okinawan sponge *T. hoshinota*.[4] In a continuation of this work, we isolated nakiterpiosin, a novel cytotoxic compound, from *T. hoshinota*. In this paper, we report the isolation and structure determination of nakiterpiosin (**1**).

The marine sponge *T. hoshinota* (30 kg), collected at Nakijin, Okinawa Prefecture, was steeped in acetone for 7 days. The extract was filtered, concentrated under reduced pressure, and extracted with ethyl acetate. The ethyl acetate-soluble portion was further partitioned between 90% aqueous methanol and hexane. The material obtained from the 90% aqueous methanol portion was successively chromatographed on silica gel and ODS silica gel, using bioassay-guided (cytotoxicity against P388 cells) fractionation. Final purification was achieved by reversed-phase HPLC to give nakiterpiosin (**1**) [0.2 mg, $IC_{50}=0.01$ μg/mL]. The details of the isolation and biological activity will be reported elsewhere.



1:**R=H** (Nakiterpiosin)
2:**R=Ac**

The molecular formula of nakiterpiosin was found to be $C_{27}H_{31}BrCl_2O_7$ (MNa^+, m/z 641.0490 Δ −1.7 mmu) by HRESIMS. The NMR data for **1** are summarized in Table 1. A detailed analysis of ^1H, ^{13}C NMR and HMQC spectra showed that **1** contained three methyl groups, four methylenes, 12 methines, eight quaternary carbons, and two protons on heteroatoms. The locations of the hydroxyl groups in **1** were determined based on the shifts observed for H-4 (δ_H 5.28 ppm to 6.20 ppm) and H-22 (δ_H 4.39 ppm to 5.77 ppm) in the ^1H NMR spectrum of the diacetate (**2**) that was prepared by acetylation (acetic anhydride/pyridine) of **1**. These results suggested that hydroxyl groups were located at C4 and C22. Furthermore, ^1H NMR of **1** showed the presence of a 1,2,3,4-tetrasubstituted benzene ring (δ_H 7.33, 7.89). A detailed analysis of the COSY and HOHAHA spectra of **1** allowed four partial structures, C1–C2, C6–C9, C15–C16 and C20–C26, to be constructed, as shown in Figure 1.

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Table 1. NMR data for nakiterpiosin in CD_3OD

Position	^1H (ppm)[a]	^{13}C (ppm)[b]	Position	^1H (ppm)[a]	^{13}C (ppm)[b]
1a	2.05 dd (12.3, 1.0)	41.7 t	14		152.3 s
1b	2.60 dd (12.3, 7.8)		15	7.33 d (8.2)	120.5 d
2	4.23 dd (7.8, 1.0)	75.1 d	16	7.89 d (8.2)	134.1 d
3a	4.09 d (11.2)	63.7 t	17		134.9 s
3b	3.11 d (11.2)		18	2.70 s	12.4 q
4	5.28 s	91.1 d	19	1.52 s	14.3 q
5		83.4 s	20	3.89 dd (10.3, 3.8)	51.5 d
6	4.70 dd (2.7, 1.4)	51.8 d	21	6.32 d (10.3)	75.2 d
7a	2.28 m	34.9 t	22	4.39 dd (8.0, 3.8)	71.2 d
7b	2.74 ddd (13.4, 2.7, 1.4)		23	3.92 ddd (8.2, 8.0, 3.7)	78.7 d
8	3.58 m	35.1 d	24a	1.71 ddd (12.8, 8.4, 8.2)	31.1 t
9	2.69 d (9.3)	63.5 d	24b	2.28 m	
10		44.8 s	25	2.69 m	33.3 d
11		204.6 s	26	1.13 d (7.3)	14.8 q
12		138.6 s	27		180.6 s
13		135.7 s			

[a] Recorded at 800 MHz. Coupling constants (Hz) are in parentheses.
[b] Recorded at 200 MHz. Multiplicity was based on the HMQC spectrum.

Furthermore, HMBC techniques revealed that these fragments were linked through crosspeaks due to $^2J_{CH}$, $^3J_{CH}$ long-range coupling with quaternary carbons. The HMBC crosspeaks H-26/C27 and H-25/C27 suggested connectivity between C25 and C27. The connections among the aromatic partial structure units were clarified by the HMBC crosspeaks: H-18/C12, H-18/C13, H-18/C17, H-16/C13, H-16/C14, H-15/C17 and H-15/C12.

The HMBC crosspeaks, H-19/C1, H-19/C5, H-19/C9, H-19/C10, H-6/C5, H-6/C10 and H-2/C5, indicated a 5,6-bicyclic ring (B,C-bicyclic ring). In addition, the B,C-bicyclic ring was linked to the A-ring to make a 6,5,6-tricyclic ring, as suggested by the HMBC crosspeaks H-2/C3, H-3/C1, H-3/C2, H-3/C4, H-4/C3 and H-4/C5. The HMBC crosspeaks H-9/C11 revealed connectivity between C9 and C11. Furthermore, the HMBC crosspeaks H-16/C20, H-20/C16 and H22/C17 suggested connectivity between C17 and C20. Allylic coupling (H-8 and H-15) and HMBC crosspeaks H-15/C8 and H-8/C14 suggested connectivity between C8 and C14. Connectivity between C11 and C12 was suggested by the chemical shift of C11, which is considered to be an α,β-unsaturated ketone. Consequently, the entire carbon chain was assembled as shown in **1**, and all protons and carbons were assigned as shown in Table 1.

The presence of a dichloromethyl group was suggested based on characteristic ^1H NMR and ^{13}C NMR signals (δ_C 75.2, δ_H 6.32).[5] Furthermore, the bromine-bearing methine carbon was considered to be C6 because of its reasonable chemical shift (δ_C 51.8, δ_H 4.70).[6,7] Thus, the gross structure of nakiterpiosin was determined to be as shown in Figure 1.

The relative stereochemistry in **1** was established by $^3J_{H-H}$ coupling constants and NOE correlations from NOESY data. The large magnitude of $^3J_{H8-H9}=9.3$ Hz

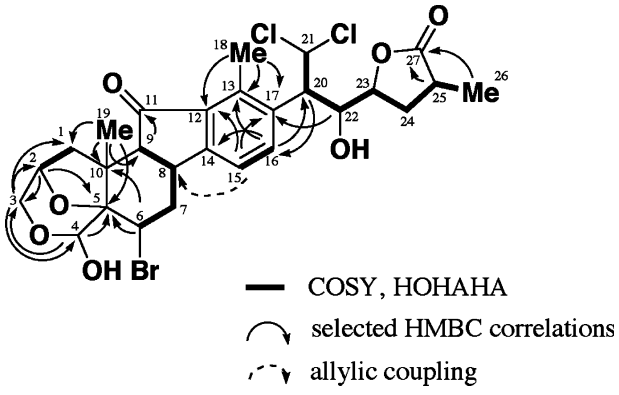

Figure 1. Structure of nakiterpiosin (**1**), based on 2D NMR correlations.

and NOESY crosspeaks 19-Me/H-8 indicated that 19-Me at C10 is in an axial position. The configuration at C4 and C6 was assigned as shown in Figure 2 based on NOE crosspeaks 19-Me/H-4 and H-4/H-6.[8] The magnitude of $^3J_{H20-H22}=3.8$ Hz and $^3J_{H22-H23}=8.0$ Hz suggested that H-20 and H-22 were located in a *gauche*

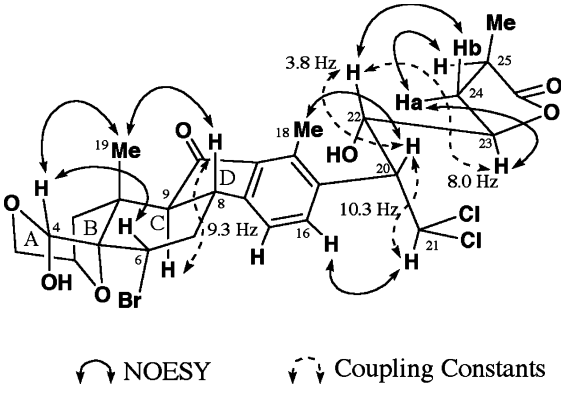

Figure 2. Relative stereochemistry of nakiterpiosin (**1**).

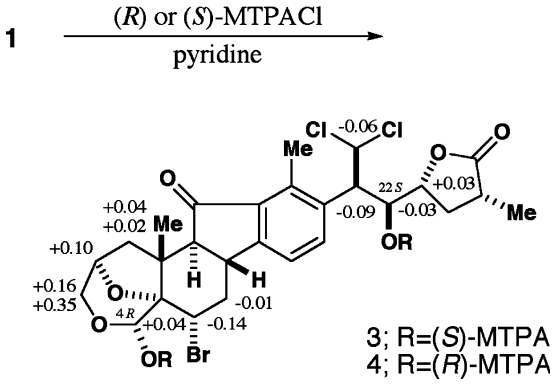

Figure 3. $\Delta\delta$ values ($\delta_S-\delta_R$) for the MTPA esters **3** and **4** in ppm.

arrangement while H-22 and H-23 were located in an *anti* arrangement. The NOE correlationships of H-16/H-21 and H-18/H20 suggested that the relative stereochemistry at C20 were 20*R**. Based on the presumption that the alkyl side chain of **1** have a zigzag conformation, we have deduced that the relative stereochemistry at C22, C23 and C25 are 22*S**, 23*R** and 21*R**. The NOE crosspeaks are consistent with this assignment. Eventually, all of the relative configurations of **1** were elucidated to be as shown in Figure 2.

The absolute stereostructure of **1** was determined as follows using a modified Mosher's method.[9] Treatment of **1** with (*R*)- and (*S*)-MTPACl gave (*S*)- and (*R*) MTPA esters **3** and **4**. The ^{1}H NMR signals of these two MTPA esters **3** and **4** were assigned on the basis of the COSY spectra, and the $\Delta\delta$ values ($\delta_S-\delta_R$, ppm) were then calculated. The results, shown in Figure 3, established that the absolute stereochemistries of C4 and C22 were 4*R* and 22*S*, respectively. Therefore, the absolute stereostructure of nakiterpiosin (**1**) was determined to be as shown in formula **1**.

In conclusion, the novel cytotoxic compound nakiterpiosin was isolated from the Okinawan sponge *T. hoshinota*. The structure of **1** was determined based on 2D NMR spectra and a modified Mosher's method. Nakiterpiosin (**1**) possesses an attractive structure consisting of a highly functionalized, oxidatively degraded C-nor-D-homosteroidal skeleton, including a dichloromethyl group in its alkyl side chain. The carbon skeleton of **1** resembles those of veratrum alkaloids.[10] These alkaloids are well-known metabolites of terrestrial plants, but have not been previously reported in marine organisms. This is the first report of C-nor-D-homosteroid produced by a marine organism. To protect corals[11] from the sponge *T. hoshinota*, it is very important to elucidate the mechanism of how this sponge kills and smothers coral communities. Since nakiterpiosin exhibited potent cytotoxicity against P388 cells, we deduce that this compound could be cytotoxic toward coral tissue. Further studies on the chemical and biological properties of nakiterpiosin are currently underway in our laboratory.

Acknowledgements

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